

**CORRECTED
VERSION***

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



(6)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A01G 1/00, A01H 1/00, 1/04, 5/10, C12N 5/04, 5/10		A1	(11) International Publication Number: WO 98/37752 (43) International Publication Date: 3 September 1998 (03.09.98)
(21) International Application Number: PCT/US98/03604		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(22) International Filing Date: 26 February 1998 (26.02.98)			
(30) Priority Data: 60/039,226 28 February 1997 (28.02.97) US			
(71) Applicant: CORNELL RESEARCH FOUNDATION, INC. [US/US]; Suite 105, 20 Thornwood Drive, Ithaca, NY 14850 (US).		Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(72) Inventors: ZITTER, Thomas, A.; 4 Bean Hill Lane, Ithaca, NY 14850 (US). WEI, Zhong-Min; 8230 125th Court, Kirkland, WA 98034 (US).			
(74) Agents: GOLDMAN, Michael, L. et al.; Nixon, Hargrave, Devans & Doyle LLP, Clinton Square, P.O. Box 1051, Rochester, NY 14603 (US).			

(54) Title: INSECT CONTROL WITH A HYPERSENSITIVE RESPONSE ELICITOR

(57) Abstract

The present invention relates to a method of controlling insects on plants. This involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to control insects on the plant or plants produced from the plant seed. Alternatively, transgenic plants or transgenic plant seeds transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to control insects.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

**INSECT CONTROL WITH A
HYPERSENSITIVE RESPONSE ELICITOR**

This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/039,226, filed February 28, 1997.

5

FIELD OF THE INVENTION

The present invention relates to the control of insects.

10

BACKGROUND OF THE INVENTION

The introduction of synthetic organic pesticides following World War II brought inestimable benefits to humanity and agricultural economic 15 profitability. The widescale deployment of DDT resulted in the complete riddance, from entire countries, of serious public pests such as malaria mosquitoes. The use of DDT, other organochlorines, and, later, organophosphorus and carbamate materials was 20 enthusiastically adopted into control programs despite occasional warnings about the hazard of unilateral approaches to pest control.

The development of new pesticides and the increasing amounts of pesticides used for pest control 25 are closely correlated with the development of pest resistance to chemicals. The number of pesticide resistant species has greatly increased since the adoption of DDT in 1948. As a result, by the 1980s, the number of reports of pesticide resistance for arthropod 30 pests was listed as 281, for plant pathogens 67, and for weeds 17. These numbers have steadily increased to the present day. Thus, the need for biological control agents, especially those with broadbase activity is especially important.

- 2 -

The present invention is directed to overcoming these problems in the art.

SUMMARY OF THE INVENTION

5

The present invention relates to a method of insect control for plants. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plants or plant seeds under 10 conditions effective to control insects on the plants or plants grown from the plant seeds.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to control insects on plants or 15 plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant 20 under conditions effective to permit that DNA molecule to control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the 25 planted seed under conditions effective to control insects.

The present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention 30 encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, 35 interfering with insect larval feeding on such plants,

WO 98/37752

- 3 -

preventing insects from colonizing host plants,
preventing colonizing insects from releasing phytotoxins,
etc. The present invention also prevents subsequent
disease damage to plants resulting from insect infection.

5 As a result, the present invention provides
significant economic benefit to growers.

BRIEF DESCRIPTION OF THE DRAWINGS

10 Figure 1 is a plot for the field study of

Example 4.

Figure 2 shows the mean number of pepper fruit
lost to bacterial soft rot for control, Kocide, Kocide +
Maneb, and hypersensitive response elicitor ("harpin")
15 treatments predisposed by European Corn Borer.

Figure 3 shows the mean number of pepper fruit
(all sizes) damaged by European Corn Borer for control,
Kocide, Kocide + Maneb, and hypersensitive response
elicitor ("harpin") treatments.

20 Figure 4 shows the mean number of large pepper
fruit damaged by European Corn Borer for control, Kocide,
Kocide + Maneb, and hypersensitive response elicitor
("harpin") treatments.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method of
insect control for plants. This method involves applying
a hypersensitive response elicitor polypeptide or protein
30 in a non-infectious form to all or part of a plant or a
plant seed under conditions to control insects on plants
or plants grown from the plant seed. Alternatively, the
hypersensitive response elicitor protein or polypeptide
can be applied to plants such that seeds recovered from
35 such plants are themselves effective to control insects.

- 4 -

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to control insects on the plants or plants grown from the seeds, transgenic plants or plant
5 seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to
10 control insects. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that
15 DNA molecule to control insects.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention can correspond to hypersensitive response elicitor polypeptides or proteins derived from a wide variety of
20 fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthomonas* species
25 (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas campestris*, and mixtures thereof).

30 An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora pythium*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*,
35 *Phytophthora megasperma*, and *Phytophthora citrophthora*.

- 5 -

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein. In addition, seeds in accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

In one embodiment of the present invention, the hypersensitive response elicitor polypeptides or proteins can be isolated from their corresponding organisms and applied to plants or plant seeds. Such isolation procedures are well known, as described in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin_{Pss}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*, Science 257:85-88 (1992), which are hereby incorporated by reference. See also pending U.S. Patent

- 6 -

Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably, however, the isolated hypersensitive response elicitor polypeptides or proteins of the present invention are 5 produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plants or plant seeds by applying bacteria containing genes 10 encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant or plant seeds cells. In these embodiments, the hypersensitive response elicitor 15 polypeptide or protein is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria do not cause 20 the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which does not elicit a hypersensitive response in plants, can be transformed with genes encoding a 25 hypersensitive response elicitor polypeptide or protein and then applied to plants. Bacterial species other than *E. coli* can also be used in this embodiment of the present invention.

In another embodiment of the bacterial 30 application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment, these bacteria are applied to plants or 35 their seeds which are not susceptible to the disease

- 7 -

carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this 5 embodiment of the present invention, *Erwinia amylovora* can be applied to tomato plants or seeds to enhance growth without causing disease in that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an 10 amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

	Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser	
	1 5 10 15	
15	Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser	
	20 25 30	
20	Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr	
	35 40 45	
	Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu	
	50 55 60	
25	Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser	
	65 70 75 80	
	Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys	
	85 90 95	
30	Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp	
	100 105 110	
35	Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln	
	115 120 125	
	Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met	
	130 135 140	
40	Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly	
	145 150 155 160	
	Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly	
	165 170 175	
45	Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu	
	180 185 190	
50	Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala	
	195 200 205	
	Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val	
	210 215 220	

- 8 -

	Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
	225 230 235 240
5	Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
	245 250 255
	Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
	260 265 270
10	Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
	275 280 285
	Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
15	290 295 300
	Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
	305 310 315 320
20	Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
	325 330 335
	Asn Ala

25 This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein
 30 is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

	CGATTTTACCGGGTGAAACGTGCTATGACC GACAGCATCA CGGTATTGCA CACCGTTACG	60
35	GCGTTTATGGCCCGATGAA CCGGCATCAG GCGGCGCGCT GGTGCGCCGCA ATCCGGCGTC	120
	GATCTGGTATTCAGTTGGGGACACCGGGCGTGAACACTCA TGATGCAGAT TCAGCCGGGG	180
40	CAGCAATATCCGGCATGTT GCGCACGCTGCTCGCTCGTC TTATCAGCA GGCGGCAGAG	240
	TGGCATGGCTGCCATCTGTG CCTGAACGGCAGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
	CCGTGGGATCCGGCAGTTA TCCGCAGGTGATCGAACGTT TGTTGAACTGGCGGGAAATG	360
45	ACGTTGCCGT CGCTATCCAT AGCACCGACGGCGTCCCGCAGACAGGGAA CGGACGCGCC	420
	CGATCATTAA GATAAAGGCG GCTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
50	CACCGTCGGC GTCACTCAGT AACAAAGTATC CATCATGATG CCTACATCGG GATCGCGTG	540
	GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGCGTC TCCGGTCTGG GGCTGGGTGC	660
55	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTGGTTCCAGCG TGGATAAACT	720
	GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
	GGCGCAGGGCTGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840

- 9 -

	TTTCGGCAAT GGCAGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCCGA	900
	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTATG ACACCGTGAC	960
5	CAAGCTGACT AACCAAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACGTGCGT CCATTCTCGG	1080
10	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	1200
	GGGGCAGAAC GCTGCGCTGA GTGCCTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
15	CCGCCACTTT GTAGATAAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
	TCAGTATCCG GAAATATTCTG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
20	GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCG	1440
	CGCCAGCATG GACAAATTCC GTCAGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
	TACCGGCAAT ACCAACCTGA ACCTGGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
25	GGCTGTCGTC GGGGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
	ATCTGTGCTG GCCTGATAAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTC	1680
30	TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
	ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGGCCACA ATCGCGATGG CATCTTCCTC	1800
	GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
35	CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTAG	1920
	CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCGTGTG CGCCGGCCGG	1980
40	GATCACCAACA ATATTCAATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
	AAAATAGGGC AGTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
	GTTCGTCATC ATCTTTCTCC ATCTGGCGA CCTGATCGGT T	2141
45		

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

50	Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser	
	1 5 10 15	
55	Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln	
	20 25 30	
	Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Asn	
	35 40 45	
60	Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met	
	50 55 60	

- 10 -

	Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65	70 75 80
5	Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
	85 90 95
	Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
	100 105 110
10	Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
	115 120 125
15	Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
	130 135 140
	Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
	145 150 155 160
20	Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
	165 170 175
	Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
	180 185 190
25	Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
	195 200 205
30	Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
	210 215 220
	Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
	225 230 235 240
35	Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
	245 250 255
	Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
	260 265 270
40	Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
	275 280 285
45	Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
	290 295 300
	Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
	305 310 315 320
50	Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
	325 330 335
	Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
	340 345 350
55	Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
	355 360 365
	Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
60	370 375 380
	Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
	385 390 395 400
65	Gly Ala Ala

- 11 -

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5	AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTGAA TTATTCATAA	60
10	GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTCT	120
15	ATCGGCCGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG	180
20	GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG	240
25	GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
30	GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
35	GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
40	GGCGGCAACA ATACCACCTTC AACAAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC	480
45	TCAACGTCAC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
50	CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG	600
55	CAAGATGGCA CCCAGGGCAG TTCCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
60	GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGTAATGG TCTGAGCCAG	720
65	CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGT AATGCTGGCAC GGGTCTTGAC	780
70	GGTCGTCGC TGGGCGCAA AGGGCTGCAA AACCTGAGCG GCCCGGTGGA CTACCAAGCAG	840
75	TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT	900
80	ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG	960
85	GCGAAGGAAA TCGGTCAGTT CATGGACCAAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC	1020
90	CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGCAAA AGCACTGAGC	1080
95	AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140
100	ATGATCAAAA GGCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200

- 12 -

GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

5

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 5 as follows:

10

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met			
1	5	10	15

15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser			
20	25	30	

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met			
35	40	45	

20

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala			
50	55	60	

25

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val			
65	70	75	80

30

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe			
85	90	95	

35

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met			
100	105	110	
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu			
115	120	125	

40

130	135	140	
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met			

45

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro			
145	150	155	160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe			
165	170	175	

50

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile			
180	185	190	

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly			
195	200	205	

55

Thr Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser			
210	215	220	

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser			
225	230	235	240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp			
245	250	255	

- 13 -

	Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
	260 265 270
5	Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
	275 280 285
	Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Lys Gly Leu Glu Ala
	290 295 300
10	Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
	305 310 320
	Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
	325 330 335
15	Asn Gln Ala Ala Ala
	340

20 This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in
 25 He, S. Y., H. C. Huang, and A. Collmer, "Pseudomonas syringae pv. syringae Harpin_{Pss}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The
 30 DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

35	ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG	60
	GTACGTCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC	120
	GTGAAGCTGG CCGAGGAACt GATGCCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA	180
40	AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCAG GCGGGCGGTAT TGAGGATGTC	240
	ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG	300
	GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC	360
45	AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC	420
	GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTCCC	480
50	AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAGGAAG ACAACTTCCT TGATGGCGAC	540
	GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG	600

- 14 -

	AGT GAC GCT G	GCAG TCT GGC	AGGG AC GGGT	GGAG GT CT GG	GCA CT CC GAG	CAG TTT TTCC	660
	AACA ACT CGT	CCGT GAT GGG	TGAT CC GCT G	ATCG AC GCCA	ATAC CCGT CC	CGGT GAC AGC	720
5	GGCA AT ACCC	GTGG TGAA GC	GGGG CA ACTG	ATCG CGAG C	TTAT CGAC CG	TG GCCT GCAA	780
	TCGG TATT GG	CCGG TG GT GG	ACT GGG CACA	CCCG TAA ACA	CCCC CGAG AC	CGGT AC GT CG	840
10	GCGA AT GGCG	GAC AGT CC GC	TCAG GGAT CT	T GAT CGAT TC	TGGG CGG CTT	GCT GCT CAAG	900
	GGC CT GGAG G	CAAC GCT CAA	GGAT GCC GGG	CAAAC AGG CA	CCG AC GT GCA	GT CGAG CG CT	960
	GCG CAA AT CG	CCAC CCTTG CT	GGTC AGT AC	CG CT GCA AG	GCAC CC GCAA	TCAG GCT GCA	1020
15	GCCT GA						1026

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

	Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln	1	5	10	15
25	Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser		20	25	30
	Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile	35	40	45	
30	Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly	50	55	60	
35	Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala	65	70	75	80
	Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser	85	90	95	
40	Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met	100	105	110	
	Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala	115	120	125	
45	Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val	130	135	140	
50	Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala	145	150	155	160
	Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly	165	170	175	
55	Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly	180	185	190	
60	Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala	195	200	205	

- 15 -

	Asp	Gly	Gly	Asn	Gly	Val	Asn	Gly	Asn	Gln	Ala	Asn	Gly	Pro	Gln	Asn	
	210						215					220					
5		Ala	Gly	Asp	Val	Asn	Gly	Ala	Asn	Gly	Ala	Asp	Gly	Ser	Glu	Asp	
		225					230					235			240		
		Gln	Gly	Gly	Leu	Thr	Gly	Val	Leu	Gln	Lys	Leu	Met	Lys	Ile	Leu	Asn
						245					250				255		
10		Ala	Leu	Val	Gln	Met	Met	Gln	Gln	Gly	Gly	Leu	Gly	Gly	Asn	Gln	
						260					265			270			
		Ala	Gln	Gly	Gly	Ser	Lys	Gly	Ala	Gly	Asn	Ala	Ser	Pro	Ala	Ser	Gly
						275					280			285			
15		Ala	Asn	Pro	Gly	Ala	Asn	Gln	Pro	Gly	Ser	Ala	Asp	Asp	Gln	Ser	Ser
						290					295			300			
20		Gly	Gln	Asn	Asn	Leu	Gln	Ser	Gln	Ile	Met	Asp	Val	Val	Lys	Glu	Val
						305					310			315		320	
		Val	Gln	Ile	Leu	Gln	Gln	Met	Leu	Ala	Ala	Gln	Asn	Gly	Ser	Gln	
											325		330		335		
25		Gln	Ser	Thr	Ser	Thr	Gln	Pro	Met								
						340											

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 8 as follows:

30	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
35	GAGAAGGACA	TCCTCAACAT	CATCGCAGCC	CTCGTGCAGA	AGGCCGCACA	GTCGGCGGGC	180
	GGCAACACCG	GTAACACCGG	CAACGCGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
40	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCGGCCGG	CAATGACAAG	420
45	GGCAACGGCG	TGGCGGGTC	CAACGGGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCGCGGGCGG	TGCTGGCGCC	540
50	GGCGCGCGG	GTGGCGGTGT	CGCGGGTGT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
	GGCCCGCAGA	ACGCAGGCCA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
55	CAGGGCGGCC	TCACCGGCGT	GCTGAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCCT	CGGCGGCCGC	AACCAGGCCG	AGGGCGGCTC	GAAGGGTGCC	840
60	GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGCGCGA	ACCAGCCCGG	TTCGGCGGAT	900
	GATCAATCGT	CCGGCCAGAA	CAATCTGCAA	TCCCAGATCA	TGGATGTGGT	GAAGGAGGTC	960
	GTCCAGATCC	TGCAGCAGAT	GCTGGCGGCCG	CAGAACGGCG	GCAGCCAGCA	GTCCACCTCG	1020
65	ACGCAGGCCA	TGTAA					1035

- 16 -

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a 5 Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor 10 polypeptide or protein from *Xanthomonas campestris* pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

15 Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
 1 5 10 15
 Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
 20 25

20 This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. glycines. It matches with fimbrial subunit proteins 25 determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid 30 sequence corresponding to SEQ. ID. No. 10 as follows:

35 Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
 1 5 10 15
 Leu Leu Ala Met
 20

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora*

- 17 -

subsp. carotovora Strain Ecc71 Overexpress *hrp N_{Ecc}* and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide is shown in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from *Phytophthora*: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi *Phytophthora* Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of *Phytophthora parasitica*," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

- 18 -

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under which genes encoding an elicitor are expressed. Cell-free 5 preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of 10 full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional 15 molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave 25 elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the 30 primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for

- 19 -

increase and expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out 5 using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of a useful fragment is the popA1 fragment of the hypersensitive response elicitor polypeptide or protein from *Pseudomonas solanacearum*. See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet, and C.A. Boucher, "PopA1, a Protein Which Induces a 10 Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-53 (1994), which is hereby incorporated by reference. As to *Erwinia amylovora*, a suitable fragment can be, for example, either or both the 15 polypeptide extending between and including amino acids 1 and 98 of SEQ. ID. NO. 3 and the polypeptide extending between and including amino acids 137 and 204 of SEQ. ID. No. 3.

Variants may also (or alternatively) be modified by, for example, the deletion or addition of 20 amino acids that have minimal influence on the properties, secondary structure and hydrophobic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or 25 post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or 30 identification of the polypeptide.

- 20 -

The protein or polypeptide of the present invention is preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the protein or polypeptide of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the polypeptide or protein of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage

- 21 -

and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eucaryotic cells grown in tissue culture.

5 Recombinant genes may also be introduced into viruses, such as vaccinia virus. Recombinant viruses can be generated by transection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited
10 to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif., which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard
15 cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be
30 utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; 35 microorganisms such as yeast containing yeast vectors;

- 22 -

mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these 5 vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events 10 control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby 15 promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, 20 procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient 25 translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the 30 protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and

Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene.

Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promoter, lac promotor, trp promotor, recA promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the lac operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as trp, pro, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector,

which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon (ATG) to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to control insects. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*,

Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

The present invention is effective against a wide variety of insects. For purposes of the present 5 invention, insects (Phylum Arthropoda, Class Insecta) also encompasses Phylum Mollusca (snails and slugs represented by the spotted garden slug, banded slug, marsh slug, and gray garden slug), Class Arachnida (mites), and Phylum Nematoda (roundworms or nematodes). 10 The host range for some of these pests is extensive. For example, the European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plants species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed 15 species. Additional insect larvae and adult feeding pests which feed on and damage a wide variety of vegetables and small fruits include the following: Vegetables -- seed corn maggot, rice armyworm, alfalfa leafhopper, aster leafhopper, beet armyworm, cabbage looper, cabbage root maggot, Colorado potato beetle, corn 20 earworm, cotton or melon aphid, diamondback moth, fall armyworm, flea beetles (various adult species feed on cabbage, mustard, and other crucifers, cucumber, eggplant, tobacco, potato, melon, and spinach), green 25 peach aphid, onion maggot, onion thrips, pepper maggot, pickleworm (melon worm), potato leafhopper, potato stem borer, potato and corn stalk borer, striped cucumber beetle, spotted cucumber beetle, northern and western corn root worm, thrips, tarnish plant bug, tobacco aphid, 30 tomato pinworm, tomato mole cricket, and rootknot nematode; Small fruits -- meadow spittlebug, strawberry bud weevil, strawberry root weevil, tarnish plant bug, and strawberry spider mites; Grapes -- grape berry moth, grape cane gallmaker, climbing cutworms, grape 35 leafhoppers (three species), and grape canc girdler. Collectively this group of insects and allied species

- 26 -

represents the most economically important group of pests for vegetables, small fruit, and grape production worldwide.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, dusting, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, dusting, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to control insects on the plants. Such propagated plants may, in turn, be useful in producing seeds or propagules (e.g., cuttings) that produce plants capable of insect control.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant

- 27 -

seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, herbicide, and mixtures thereof. Suitable fertilizers include $(\text{NH}_4)_2\text{NO}_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well known in the art, such as by

biolistics or *Agrobacterium* mediated transformation. Examples of suitable hypersensitive response elicitor polypeptides or proteins and the nucleic acid sequences for their encoding DNA are disclosed *supra*. Once 5 transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in control of insects on the plant. Alternatively, 10 transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions 15 effective to control insects. While not wishing to be bound by theory, such growth enhancement may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used 20 in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including 25 hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, dusting, and immersion. Similarly, after plants have been propagated from the transgenic 30 plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to control insects. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.). The transgenic plants of the present 35 invention are useful in producing seeds or propagules

- 29 -

(e.g., cuttings) from which plants capable of insect control would be produced.

EXAMPLES

5

Example 1 - Controlling the Spread of Aphids From Colonized or Infested Tobacco

Two to three lower leaves (at position 4) of a 10 tobacco plant were infiltrated with hypersensitive response elicitor at a concentration of 20 $\mu\text{M}/\text{ml}$. Another tobacco plant infiltrated with 5 mM potassium phosphate buffer was used as a control. Any visible aphids on these two plants were then killed. The two 15 plants were placed on a lab bench with a light on at night. Five days after infiltration of hypersensitive response elicitor, a heavily aphid-infected tobacco plant was moved from the greenhouse to the lab bench. The aphid-infected plant was placed close to and between the 20 hypersensitive response elicitor-treated plant and the buffer-infiltrated plant with many of the leaves of the uninfected plants overlapping with those of the infected plant to facilitate movement of the aphids from the infected plant. The number of aphids on hypersensitive 25 response elicitor- and buffer-treated plants were counted once everyday for about 10 days. The result is shown in Table 1.

Table 1 - Harpin Induced Tobacco Resistance To Aphid Infection

	A	B	C	D	E	F	G	H	I
	Leaf Position	Day 1	Day 2	Day 3	Day 6	Day 7	Day 8	Day 9	Day 10
	H C	H C	H C	H C	H C	H C	H C	H C	H C
1	7 5	17 9	8 7	8 9	7 11	4 13	10 22	17 32	
2	3 7	12 5	12 19	8 12	24 39	22 39	17 26	4 22	
3	3 7	3 12	3 27	1 >50	4 >50	12 >50	2 >50	4 >50	
4	4 10	3 12	2 >50	1 >50	0 >50	0 >50	0 >50	0 >50	
5	2 6	1 8	0 10	0 18	0 22	0 22	0 22	0 22	
6	2 0	2 4	0 4	0 11	0 22	0 20	0 18	1 26	
7	0 0	0 0	0 4	0 14	0 14	2 14	0 10	0 10	
8	1 12	1 4	0 8	1 24	0 22	0 22	0 32	0 32	
9	0 0	0 5	0 5	0 7	0 12	0 9	0 9	0 7	
10	0 0	0 3	0 3	0 13	0 15	0 15	0 12	0 10	
11	0 0	0 5	0 5	0 6	0 11	0 11	0 11	0 19	
12	0 2	0 3	0 11	0 11	0 6	0 6	0 8	0 8	
13	0 0	0 0	0 0	0 2	0 2	0 6	0 6	0 6	
14	0 0	0 0	0 0	0 1	0 6	0 8	0 21	0 14	
15	0 0	0 0	0 7	0 9	0 32	0 32	0 32	0 22	
16	0 0	0 0	0 0	1 0	0 0	0 2	0 4	0 11	
17	0 0	0 0	0 0	0 1	0 5	0 5	0 4	0 14	
18	0 0	2 0	0 0	0 1	0 1	0 1	0 1	0 4	
19	1 11	1 11	0 8	4 17	4 17	0 17	2 13	0 7	
20	0 0	0 0	0 0	0 0	0 0	0 0	4 6	4 14	
Total	23 60	42 81	25 >169	24 >260	39 >337	38 >341	37 >388	34 >409	

H: Harpin-induced plant
C: Control plant

- 31 -

From these results, it is clear that the hypersensitive response elicitor-treated plant has many fewer aphids than the buffer-treated control plant, suggesting that the aphids did not like to colonize on the hypersensitive response elicitor-treated plants. At the lower three leaves, there was a substantial number of aphids even in the hypersensitive response elicitor-treated plant. Since infiltration of hypersensitive response elicitor started from leaf 4, this indicates that the hypersensitive response elicitor-generated signal for insect-resistance can only effectively travel upward to the top of the tobacco plant.

It was also observed that aphids died 2 days after they moved to the hypersensitive response elicitor-treated plant.

Example 2 - Colonization of Aphids in Hypersensitive Response Elicitor-Treated Tobacco Plants

From Example 1, it was observed that there were many dead aphids on the hypersensitive response elicitor-treated tobacco leaves. To further confirm this observation, aphids were artificially inoculated on a hypersensitive response elicitor-treated tobacco plant. The number of living and dead aphids were counted once every day for 4 days.

Hypersensitive Response Elicitor Treatment and Aphid Inoculation: Two lower leaves of tobacco plants were infiltrated with hypersensitive response elicitor at a concentration of 20 µg/ml. After 24 hours, tissue necrosis was observed. Seven days after hypersensitive response elicitor infiltration, aphids from an infested (or colonized) plant were transferred to the three upper leaves of the hypersensitive response elicitor-treated plant.

- 32 -

Table 2 summarizes the results of this example. It shows that, after two days, most of the inoculated aphids were dead and some of them moved away from the hypersensitive response elicitor-treated plant; however, 5 the number of the inoculated aphids in the control plant remained about the same.

Table 2 - Number of Colonized Aphids in Control and Harpin-Treated Tobacco Plants
10

	A	B	C	D	E	F
	Leaf	Day 0	Day 1	Day 2	Day 3	Day 4
		H C	H C	H C	H C	H C
15	1	23 22	18 20	6 20	0 19	0 21
	2	26 27	14 27	3 25	0 25	0 28
	3	31 25	12 26	2 22	1 24	0 20
	Total	80 74	44 73	11 67	1 68	0 69

20 The numbers in the table are live aphids

H: Harpin-induced plant

C: Control plant

25 Example 3 - Tobacco Seedlings Generated from Harpin-Soaked Seeds are Resistant to Aphid Infection

About 80 tobacco seeds (*Nicotiana tabacum* L. 'Xanthi') were soaked in harpin solution (about 25 µg/ml 30 of 5 mM potassium phosphate buffer, pH 6.5) for about 16 hours. Then, the harpin-soaked seeds were sowed in a 6" pot with artificial soil. The same treatment using a 5 mM potassium phosphate buffer without harpin was used as a control. The pots were incubated in a growth 35 chamber at a temperature of 25°C with 14 hour day light. Twenty days after sowing, the size of the tobacco seedlings treated with harpin was significantly greater

- 33 -

than that of control plants. Twenty seedlings subjected to each treatment were transplanted to 8" pots 28 days after sowing. The seedlings were then incubated in a growth room at a temperature of about 23°C using 14 hour 5 day lights. By the time the seedlings were transplanted, aphid infection was observed in the control tobacco seedlings, but not in the harpin-treated seedlings. The source of aphid infection was previously infected adult tobacco plants in the same growth chamber. In the growth 10 room, 7 precolonized adult tobacco plants were placed around the seedlings being tested to serve as a natural source of aphids. Seven days after the seedlings were transplanted, the number of aphids in each tobacco seedlings was counted. As shown in Table 3, 17 out of 20 15 control plants were infected by aphids with the number of aphids varying between 1 to 13. However, only 2 out of 20 harpin-treated plants were infected by the aphids. This indicates that tobacco plants from harpin-treated seeds are far more resistant to the aphid infection than 20 control plants.

- 34 -

Table 3 - Tobacco Plants Generated From Harpin-Soaked Seeds Are Resistant To Aphid Infection

5	<u>Plant No.</u>	<u>Control</u>		<u>Harpin-Treated</u>	
		<u>Number of Aphids</u>	<u>Plant No.</u>	<u>Number of Aphids</u>	<u>Plant No.</u>
10	1	4	1	0	
	2	2	2	10	
	3	11	3	0	
	4	11	4	0	
	5	4	5	0	
	6	13	6	0	
	7	3	7	0	
	8	5	8	0	
	9	11	9	0	
15	10	1	10	0	
	11	3	11	0	
	12	4	12	0	
	13	4	13	0	
	14	0	14	0	
20	15	12	15	0	
	16	2	16	0	
	17	0	17	0	
	18	2	18	0	
	19	0	19	0	
25	20	2	20	0	
	Total	94		10	

Example 4 - Field Study Regarding The Effect Of Hypersensitive Response Elicitor Application On Insect Control

5 An experiment was conducted at the Homer C. Thompson Vegetable Research Farm located in Freeville, NY. The experimental design was a randomized complete block with four replications, with 8 plants per rep, using single rows on plastic, with 22 inch spacing

10 between plants. A single inoculated spreader row of peppers ran the length of the plot between the two treatment rows to provide inoculum for the target disease of bacterial leaf spot of pepper (*Xanthomonas campestris* pv. *vesicatoria*, pepper race). See Figure 1 . Upwind

15 and across the road from the pepper trial was a commercial field of dent corn which provided a natural source of European corn borer during the season. The pepper variety "Jupiter" was selected because of its strong susceptibility to bacterial leaf spot. Pepper

- 35 -

seedlings were transplanted to the field on day 0. Bacterial inoculum was introduced into the plot by two means. Previously infected "Jupiter" seedlings were transplanted to the spreader row on day 26 and the 5 spreader row was additionally inoculated on day 38 with *Xanthomonas campestris* pv. *vesicatoria* pepper race in order to provided more disease pressure for the peppers rows on either side.

The first application of hypersensitive 10 response elicitor or harpin was made on day 23, before any inoculum was introduced or spread had occurred. A total of four treatments were tested: (1) water sprayed control; (2) Kocide at 3 lb/A; (3) Kocide at 1 lb + Manex fungicide at 1.2 qt/A; and (4) Harpin. The copper 15 fungicide Kocide and the Kocide + Manex (maneb) fungicide are standard materials recommended for bacterial leaf spot control in pepper. Kocide is manufactured by Griffin Corp., Valdosta, GA, while Manex is produced by Crystal Chemical Inter-Americal, Houston, TX. All 20 treatments were applied with a CO₂ pressurized boom sprayer at approximately 40 psi with 21.5 gal/A being delivered through four TeeJet XR 11003 flat fan nozzles spaced 20 inches apart. This provided excellent foliar coverage. Following initial harpin treatment, all 25 treatments were applied weekly until the experiment was concluded. No additional pesticides, including insecticides, were applied. The first appearance of disease in the test plants was on day 54. Two pepper harvests were made on day 61 and day 97. Data taken 30 included the incidence (i.e. number of plants infected with bacterial leaf spot) per treatment, total number and weight of fruit harvested by category (large, medium, small, and unmarketable), and the total number of fruit showing European corn borer damage expressed as frass or 35 unharvestable because of fruit breakdown by bacterial

soft rot *Erwinia carotovora* subsp. *carotovora*. The involvement of European corn borer became evident at about day 50. Consequently, the amount of soft rot for all treatments was recorded at the day 57 and day 97 5 harvests. Similarly, it became apparent on the day 57 harvest that European corn borer damage could also be assessed by larval feeding (i.e. frass) on pepper fruit. The European corn borer overwinters as the last larval instar, and, in the spring, the larvae pupate. Adults 10 from the multi-generation strain emerge in late May to early June and again in August. If a single generation strain is present, then the emergence will peak in July. However, in some fields of the Northeast, single and multi-generation strains may be present together. Female 15 moths fly into susceptible crops to lay their eggs, and each female may lay up to 500 eggs during its lifespan. After hatching, the tiny borers crawl to protected areas on the plant to feed, which in the case of pepper, is under the calyx attachment of the pod to the stem. They 20 later bore into the pod, allowing bacteria to enter and rapidly multiply in the moist and humid environment within the pod. Bacterial soft rot can destroy the pod in a manner of days. Differences in European corn borer damage and infestations among treatments was recorded at 25 the time of the second harvest. Data were analyzed and significance established by one-way analysis of variance.

Bacterial leaf spot foliar infections occurred throughout the plots, but the amount of disease did not allow for any significant differences. Final disease 30 ratings were made on day 97. The harpin treatment provided control equivalent to the commercial treatments of Kocide or Kocide + Manex, and all were better than the water-sprayed control. The number of European corn borer (ECB) damaged fruit that were rotting on the plants on 35 day 97 were recorded; they could not be harvested because

- 37 -

of watery collapse. The harpin treated plots had fewer rotting pepper pods, and although not significantly different from the other treatments ($P=0.229$), the amount of protection provided with the harpin sprays was evident
5 (See Figure 2). Another indication of the amount of damage caused by European corn borer feeding was the number of fruit showing feeding damage or frass. The harpin treated fruit had substantially less fruit damage across all fruit sizes ($P=0.076$), when compared with all
10 other treatments (See Figure 3). The number of large fruit with borer damage was significantly reduced ($P=0.048$) when sprayed with harpin (See Figure 4).

The benefit of using harpin to reduce the damage caused by the European corn borer was reflected in
15 two ways. First, substantially less bacterial soft rot leading to loss of fruit in the field was noted when harpin was applied weekly. Secondly, the number of fruit with direct borer feeding (i.e. frass) was much lower in harpin treated plots than all other treatments. The
20 greatest impact of harpin treatment on economic factors was the greater production of undamaged fruit across all size categories, and the greater yield of healthy large fruit which have the highest dollar value.

25 Example 5 - Control of Aphid from Foliar Application of HP-1000™ Hypersensitive Response Elicitor to Cotton.

Cotton aphids (*Aphis gossypii*) leave a
30 "honeydew" deposit that contaminates the lint and reduces crop value. A field trial to determine the effect of HP-1000™ Hypersensitive Response Elicitor from *Erwinia amylovora* (Eden Bioscience Corp., Bothell, Wash.) on cotton (var. Acala) was seeded in replicated (4X) plots
35 (3.2 x 25 feet) in a randomized complete block design. Treatments were HP-1000™ at 20, 60, and 80 µg/ml (a.i.) and a chemical insecticide, Asana XL® (DuPont Agricultural

- 38 -

Products, Wilmington, DE), at 8 oz./ac. Foliar treatments were applied beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a back-pack sprayer. Aphid counts were made immediately 5 prior to spray applications at 14, 28, 35, and 42 days after the first treatment (DAT 1). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates, and ten leaves per plot at the final sampling date.

10 At 14 DAT 1 (i.e. on day 14), aphid counts were relatively low across all treatments, but by 28 DAT 1 (two sprays applied) (i.e. on day 28) the number of aphids per leaf were significantly greater in Asana XL® treated plots compared to the HP-1000™ treated plots 15 (Table 4). By 35 DAT 1 (three sprays applied) (i.e. on day 35), aphid counts had risen for all treatment rates, yet aphid counts per leaf was still significantly lower for HP-1000™ treated cotton compared to the Asana XL® treatment. Finally, at 42 DAT 1 (four sprays applied) 20 (i.e. on day 42), the number of aphids per leaf had increased to a level that threatened to overwhelm all treatments, including the chemical standard insecticide. At this point, Pravado® aphicide (Bayer Corporation, Agricultural Division, Kansas City, MO) was applied to 25 all plots to eradicate aphids from all treatments and the trial was continued for crop yield only.

These data indicate that cotton treated with HP-1000™ deterred light to moderate aphid pressure and that this effect was significantly better than a standard 30 chemical insecticide, Asana XL®.

- 39 -

Table 4 - Aphid Count per Leaf on Cotton After Treatment with Asana XL[®] or HP-1000[™]

		Number of Aphids Per Leaf ¹ No. Sprays Applied/Days After Treatment			
Treatment	Rate ²	1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL [®]	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000 [™]	20 µg/ml	0.2 a	7.8 b	22.9 b	322.1 a
HP-1000 [™]	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000 [™]	80 µg/ml	0.0 a	2.7 b	25.8 b	510.2 a

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. ²Rate for Asana XL is for formulated product, rate for HP-1000[™] is for active ingredient (a.i.).

Example 6 - Control of Strawberry Spider Mites by Foliar Application of HP-1000[™] to Cotton.

Mites cause foliar damage to cotton thus reducing potential crop yield. To assess potential mite control of HP-1000[™], cotton (var. Acala) was seeded in replicated (4X) field plots (3.2 x 25 feet) in a randomized complete block field trial. Treatments included HP-1000[™] at 20, 60, and 80 µg/ml and a chemical insecticide for mites, Zephyr[®] (Novartis, Greensboro, NC), at 6 oz./ac. HP-1000[™] treatments were applied at 14 day intervals using a back-pack sprayer beginning when the crop was at three true leaves. Zephyr[®] was applied once, on the same date as the first application of HP-1000[™]. A pretreatment evaluation for strawberry spider mites (*Tetranychus turkestanii*) was made immediately before the first spray and again at 4, 7, 14, and 28 days after the first treatment (DAT 1).

Mite populations were determined by collecting twenty-five randomly chosen cotton leaves per plot. All leaves were brushed with a mite brushing machine and dislodged mites were uniformly distributed onto a

- 40 -

rotating glass plate, pretreated with a wetting agent to which they adhered. The number of motile adult mites were counted under a 30X binocular microscope. This figure was then converted to a per leaf unit.

5 A count of living or motile adult mites per leaf at the five assessment times did not appear to show significant treatment effects at any of the evaluation times (Table 5).

10 Table 5 - Number of Adult Motile Mites per Leaf After Treatment with Zephyr® or HP-1000™.

Treatment	Rate ²	Number of motile mites per leaf				
		evaluation timing				
0DAT1	4DAT1	7DAT1	14DAT1	28DAT1		
Zephyr®	6 oz/ac	3.4	0.2	0.4	0.0	0.0
HP-1000™	20 µg/ml	2.0	0.6	0.3	0.0	0.0
HP-1000™	60 µg/ml	3.7	0.5	0.1	0.2	0.1
HP-1000™	80 µg/ml	3.0	1.4	0.4	0.0	0.0

15 20 ¹Rate for Zephyr® is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

25 However, using the method of Henderson et al., "Tests With Acaracides Against Brown Wheat Mites," J. Econ. Ent. Vol. 48(2):157-61 (1955), which is hereby incorporated by reference ("Henderson"), to calculate percent mortality 30 revealed the mite control was different between treatments. (Table 6).

- 41 -

Henderson's Method is defined as:

$$\frac{Ta \times Cb}{Tb \times Ca} \times 100$$

5 Percent Mortality = 1 -

where; Ta = Number of motile mites counted after treatment,

10 Tb = Number of motile mites counted prior to treatment,

15 Ca = Number of mites in the control (check) after treatment of the test plots, and

15 Cb = Number of mites in the control (check) plot before treatment of the test plots.

20 When percent mortality was calculated at 4 DAT 1, mite control from treatment with HP-1000™ was over two times greater compared to Zephryr® (Table 6). By 7 DAT 1, mite control was still substantially better from HP-1000™ than for Zephyr®. At 14 DAT 1, mite control for HP-1000 at 80 µg/ml reached its maximum at just under 84%, roughly comparable to that seen for the Zephyr® treatment. For the remaining 14 days, mite control by 30 HP-1000™ treatments tended to decline relative to the Zephyr®. Treatment with Zephyr® reached 100% mite control by 28 DAT 1 (Table 6).

- 42 -

Table 6 - Control of Motile Adult Mites on Cotton from Treatment with HP-1000™ as Measured by Henderson's Method.

Treatment	Rate ²	Percent control of motile mites ¹			
		evaluation timing			
		4DAT1	7DAT1	14DAT1	28DAT1
HP-1000™	20 µg/ml	56.6	76.5	68.4	66.7
HP-1000™	60 µg/ml	57.1	50.0	78.5	40.0
HP-1000™	80 µg/ml	53.6	77.9	83.8	60.0
Zephyr®	6 oz/ac	28.0	66.7	89.9	100.0

¹Percent control calculated using Henderson's method (1955).

²Rate for Zephyr® is for formulated product, rate for HP-1000™ is for active ingredient (a.i.).

15

These data indicate that the mode of action for mite control is different between HP-1000™ and Zephyr®. Complete control by treatment with Zephyr® was not achieved until 28 DAT. Weekly treatments with HP-1000™ resulted in relatively "steady" mite control throughout the 28 day evaluation period. This suggests HP-1000™ may trigger an internal insect resistance process fundamentally different than chemical insecticide activity.

20

Example 7 - Reduced Feeding Activity of Mole Cricket in Tomato from Foliar Application of HP-1000™

25

Fresh market tomatoes (var. Agri-set) were planted at 12-inch spacing in 25 foot rows replicated 5 times in a randomized completed block design field trial. This disease control trial was not specifically designed 30 to assess insect resistance from treatment with HP-1000™. Foliar applications of HP-1000™ at 20 and 40 µg/ml were applied beginning at first true leaves and repeated at 7 day intervals for 8 sprays. Additional treatment included a standard commercial fungicide mixture (Bravo®

35

- 43 -

(Zeneca Ag Products, Wilmington, DE) + Manex™ + Kocide®) for control against bacterial blight disease. After the first four sprays were applied, a field evaluation was made to determine and the number of plants damaged 5 (girdled) by feeding of mole cricket (*Scapteriscus vicinus, scudder*). Data presented in Table 7 indicates that HP-1000™ treated plants had considerably less girdling from mole cricket feeding. Continued evaluations of this trial were not possible due to 10 complete crop loss from virus infection.

Table 7 - Reduced Stem Girdling of Tomatoes by Mole Cricket from Application of HP-1000™.

15

Treatment	Rate ¹	No. Plant girdled ²	% chg. Vs. UTC
UTC	-----	15	-----
Bravo®	1 quart/ac	12	-20
+Manex™	2 lbs/ac		
+Kocide®	1.5 pints/ac		
HP-1000™	40 µg/ml	4	-73
HP-1000™	40 µg/ml	7	-53

25

¹Rates for Bravo®, Manex® and Kocide® are for formulated product; rates for HP-1000 are for a.i.

²Average number of plants from 50 plants per replicate.

30

Example 8 - Reduced Feeding Activity of Army Worm in Rice from Foliar Application of HP-1000™

Rice seed (var. M-202) was presoaked for 24 35 hours in a solution of HP-1000™ at a concentration of 20 µg/ml (a.i.). Treated rice was then seeded into randomized (5X) field plots 10 x 15 feet. An untreated control treatment was also included; no foliar sprays were applied to this trial. Observation at 41 days after 40 planting revealed significant damage to leaves due to feeding of armyworm (*Spodoptera praefica*) larvae. To

- 44 -

quantify the damage, one hundred randomly selected tillers were taken from HP-1000™ treated as well as untreated plots. Samples were ranked for damage according to the following scale:

5

- | | |
|----|---|
| 10 | 1 = no tiller leaves damaged |
| | 2 = one tiller leaves with feeding damage |
| | 3 = two tiller leaves with feeding damage |
| | 4 = three tiller leaves with feeding damage |
| | 5 = four or all tiller leaves with feeding damage |

15

- Results from these rankings were then analyzed for treatment differences. Data presented in Table 8 indicate that rice plants treated with HP-1000™ had significantly less feeding damage than the UTC plants.
- 20 HP-1000™ treated rice was virtually untouched by armyworm feeding.

Table 8 - Reduced Armyworm Feeding on Rice After Seed Soak Treatment with HP-1000™.

25

Treatment	Rate ¹	Median Rating ²
UTC		3 (two tiller leaves damaged)
HP-1000™	20 µg/ml	1 (no tiller leaves damaged)

30

¹Rate is for active ingredient applied (a.i.). ²Difference in median values among the two groups is statistically different according to Mann-Whitney Rank Sum Test, P = 0.0001.

35

Example 9 - Reduced Feeding Activity of Aphids in Tobacco from Foliar Application of HP-1000™

40

Tobacco seedlings were treated with two foliar sprays of HP-1000™ at rates of 15, 30, and 60 µg/ml (a.i.). The first application was made to seedlings, the second approximately 42 days later after transplanting

- 45 -

into replicated (3X) field plots. Two days after the second application, counts for tobacco worm and aphid were made. Data presented in Table 9 illustrate that HP-1000™ treatment substantially reduced the amount of feeding activity from both tobacco worm and aphid.

Table 9 - Reduced Feeding Activity of Tobacco Worm and Aphid from Treatment with HP-1000™ on Tobacco.

10

Treatment	Rate	No. tobacco worms/100 plants	Percent of plants with aphids feeding
UTC		20	13
HP-1000™	15 µg/ml	10	7
HP-1000™	30 µg/ml	4	4
HP-1000™	60 µg/ml	10	7

20 Example 10 - Tomato Seedlings Treated with HP-1000™ Show Tolerance to Nematodes.

Tomato seedlings (var. Rutgers) were germinated in flats and grown for four weeks before transplanting into pots, two plants per pot, replicated eight times. At transplanting, seedlings were treated with HP-1000™ at 25 µg/ml via root soaking. One week after transplanting, each pot was inoculated with approximately 10,000 root knot nematode, RKN, (*Meloidogyne hapla*) eggs.

25 Thereafter, weekly root drenches of HP-1000™ continued until four weeks. After four weeks, one plant in each pot was evaluated for root weight and the number of galls (i.e. infections sites on the roots from nematode parasitism). The remaining plants were then treated with four weekly foliar sprays of HP-1000™ (25 µg/ml a.i.).

30 After all treatments had been applied, these plants were then evaluated for root weight, shoot weight, and number of fruit per plant. Four weeks after inoculation, the

- 46 -

number of galls per plant was slightly higher for HP-1000™ treated plants than for the control plants, yet the shoot weight was significantly greater for HP-1000™ treated plants (Table 10).

5

Table 10 - Number of Galls and Shoot Weight of RKN-inoculated Tomatoes After Treatment with HP-1000™.

10

Treatment	Rate ¹	No. Galls/plant	Shoot wt. ² (g/plant)
UTC	-----	427	32.8 a
HP-1000™	25 µg/ml	507	39.5 b

15

¹Rate is for amount of active ingredient, a.i. ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

20

This indicated that even though nematodes were infecting HP-1000™ treated plants, plant growth was still enhanced by the HP-1000™ treatment. Eight weeks after inoculation, (four additional foliar HP-1000™ sprays applied) shoot weight was still significantly higher for HP-1000™ treated plants vs. control plants also inoculated with RKN and the average number of fruit per plant was numerically higher in the HP-1000™ treated plants (Table 11).

25

HP-1000™ treated plants vs. control plants also inoculated with RKN and the average number of fruit per plant was numerically higher in the HP-1000™ treated plants (Table 11).

30

Table 11 - Average Shoot Weight and Average Number of Fruit per Plant of RKN-inoculated Tomatoes After Treatment with HP-1000™.

35

Treatment	Rate ¹	Shoot wt. ² (g/plant)	No. Fruit/plant
UTC	-----	69.9 a	0.875
HP-1000™	25 µg/ml	89.8 b	1.25

40

¹Rate is for amount of active ingredient, a.i. ²Means followed by different letters are significantly different according to Duncan's MRT, P=0.05.

- 47 -

These results indicate that treatment with HP-1000™ appears to enable the tomato plants to "tolerate" the negative impact of the nematodes.

5 Example 11 - Effect of *Erwinia amylovora* Hypersensitive Response Elicitor on Repellency of Cucumbers to Striped Cucumber Beetles.

The hypersensitive response elicitor protein encoded by the *hrpN* gene of *Erwinia amylovora* ("harpin") was produced by fermentation of the cloned gene in a high-expression vector in *Escherichia coli*. High-pressure liquid chromatography analysis of the cell-free elicitor preparation was used to determine its harpin content. Treatment dilutions were prepared in water. Harpin was applied as a foliar spray to caged, cucumber plants, Marketmore 76, lot #1089, to assess its ability to repel the striped cucumber beetle, *Acalymma vittatum* (Fabricius). Harpin from *E. amylovora* was applied in water at 0, 5, and 10 mg/l to cucumber plants 21-days after sowing seed in the greenhouse (plants had both cotyledons and 6-8 fully expanded leaves/plant). Each concentration was applied to three plants per block, and the treatments were replicated three times. Seven days after treatment, a mean of 4.6 adult beetles per plant were introduced manually. The insects were allowed to feed for 7 days before feeding damage to the plants was evaluated. The number of cotyledons and the number of leaves showing any damage from beetle feeding was determined. A rating scale of 0-6 (where 0 = no obvious feeding; 1 = < 15% damage; 2 = < 25% damage; 3 = < 50% damage; 4 = > 50% damage; 5 = > 75% damage; and 6 = leaf desiccated or dead due to feeding) was used to estimate the extent of damage from beetle feeding on the cotyledons and leaves.

Table 12 summarizes the effect of hypersensitive response elicitor protein concentration on

- 48 -

insect damage. The mean percent of damaged cotyledons was in direct proportion to the harpin concentration, whereas the damage to leaves was inversely proportional to harpin concentration.

5

Table 12 - Effect of Treating Cucumber Foliage with a Hypersensitive Response Elicitor on the Subsequent Feeding Damage Caused by the Striped Cucumber Beetle (*Acalymma vittatum* [Fabricius]).

10

20

25

30

35

40

Nine plants per treatment in three blocks of three each. Damage was assessed on a 0-6 scale where 0 = no feeding injury, and 6 = cotyledons and leaves dead because of extensive beetle feeding.

More damage probably occurred on the lower cotyledons, because most of the foliar harpin spray was directed to the upper foliage and it was assumed that more harpin activity would be found in the upper leaves (upward or systemic harpin effect). The cotyledons were thus very attractive for beetle feeding. Less damage occurred on leaves of plants that had been treated with the higher concentration of harpin. Thus, the effectiveness of the treatment on leaves increased as the harpin concentration increased.

The effect of harpin is significant for two reasons: 1) damage from beetle feeding on cucurbits, especially cucumbers, melons, pumpkins, and summer and winter squash, is reduced, because treatment of cucumber

		Leaves		
	Harpin Concentration (mg/l)	Cotyledons ¹ Percent Damaged	Percent Damaged	Damage Rating
	0	34	42	5.42
	5	50	18	3.40
	10	67	5	3.20

- 49 -

with harpin resulted in the plants becoming less attractive (repulsive or repellent) to insect feeding and 2) damage from the bacterial wilt disease is likely to be reduced because these same beetles vector the bacterium 5 responsible for the disease. By preventing feeding, transmission of the bacterium responsible for the disease could be reduced or eliminated. This study shows that harpin may be used to decrease insect damage caused by beetle feeding. Thus, the number of applications of 10 insecticides to particularly insect-sensitive cucurbits might be reduced or eliminated with harpin.

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and 15 variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

- 50 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Cornell Research Foundation, Inc.
- (ii) TITLE OF INVENTION: INSECT CONTROL WITH A HYPERSENSITIVE RESPONSE ELICITOR
- (iii) NUMBER OF SEQUENCES: 10
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Nixon, Hargrave, Devans & Doyle LLP
 - (B) STREET: P.O. Box 1051, Clinton Square
 - (C) CITY: Rochester
 - (D) STATE: New York
 - (E) COUNTRY: U.S.A.
 - (F) ZIP: 14603
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 60/039,226
 - (B) FILING DATE: 28-FEB-1997
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Goldman, Michael L.
 - (B) REGISTRATION NUMBER: 30,727
 - (C) REFERENCE/DOCKET NUMBER: 19603/1522
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: (716) 263-1304
 - (B) TELEFAX: (716) 263-1600

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 338 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
 1 5 10 15
 Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
 20 25 30
 Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
 35 40 45
 Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
 50 55 60
 Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
 65 70 75 80
 Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
 85 90 95
 Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
 100 105 110
 Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
 115 120 125
 Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
 130 135 140
 Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
 145 150 155 160
 Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
 165 170 175
 Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
 180 185 190
 Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
 195 200 205
 Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
 210 215 220
 Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
 225 230 235 240
 Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
 245 250 255
 Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
 260 265 270
 Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
 275 280 285
 Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
 290 295 300

- 52 -

Asn	Leu	Asn	Leu	Arg	Gly	Ala	Gly	Gly	Ala	Ser	Leu	Gly	Ile	Asp	Ala
305				310					315				320		
Ala	Val	Val	Gly	Asp	Lys	Ile	Ala	Asn	Met	Ser	Leu	Gly	Lys	Leu	Ala
	325				330				335						
Asn Ala															

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2141 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CGATTTTACC	CGGGTGAACG	TGCTATGACC	GACAGCATCA	CGGTATTGCA	CACCGTTACG	60
GCGTTTATGG	CCGCGATGAA	CCGGCATCAG	GCGGCGCGCT	GGTCGCCGCA	ATCCGGCGTC	120
GATCTGGTAT	TTCAGTTGG	GGACACCGGG	CGTGAACTCA	TGATGCAGAT	TCAGCCGGGG	180
CAGCAATATC	CCGGCATGTT	GCGCACGCTG	CTCGCTCGTC	GTTATCAGCA	GGCGGCAGAG	240
TGCGATGGCT	GCCATCTGTG	CCTGAACGGC	AGCGATGTAT	TGATCCTCTG	GTGGCCGCTG	300
CCGTCGGATC	CCGGCAGTTA	TCCGCAGGTG	ATCGAACGTT	TGTTGAACT	GGCGGGAATG	360
ACGTTGCCGT	CGCTATCCAT	AGCACCGACG	GCGCGTCCGC	AGACAGGGAA	CGGACGCGCC	420
CGATCATTAA	GATAAAGGGC	GCTTTTTTA	TTGCAAAACG	GTAACGGTGA	GGAACCGTTT	480
CACCGTCGGC	GTCACTCAGT	AACAAGTATC	CATCATGATG	CCTACATCGG	GATCGCGCTG	540
GGCATCCGTT	GCAGATACTT	TTGCGAACAC	CTGACATGAA	TGAGGAAACG	AAATTATGCA	600
AATTACGATC	AAAGCGCACA	TCGGCGGTGA	TTTGGGCGTC	TCCGGTCTGG	GGCTGGGTGC	660
TCAGGGACTG	AAAGGACTGA	ATTCCCGGGC	TTCATCGCTG	GGTTCCAGCG	TGGATAAACT	720
GAGCAGCACC	ATCGATAAGT	TGACCTCCGC	GCTGACTTCG	ATGATTTG	GGGGCGCGCT	780
GGCGCAGGGG	CTGGGCGCCA	GCTCGAAGGG	GCTGGGGATG	AGCAATCAAC	TGGGCCAGTC	840
TTTCGGCAAT	GGCGCGCAGG	GTGCGAGCAA	CCTGCTATCC	GTACCGAAAT	CCGGCGCGGA	900
TGCGTTGTCA	AAAATGTTG	ATAAAGCGCT	GGACGATCTG	CTGGGTCTG	ACACCGTGAC	960
CAAGCTGACT	AACCAGAGCA	ACCAACTGGC	TAATTCAATG	CTGAACGCCA	GCCAGATGAC	1020
CCAGGGTAAT	ATGAATGCGT	TCGGCAGCGG	TGTGAACAAC	GCACTGTCGT	CCATTCTCGG	1080

- 53 -

CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGCGT	1200
GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAAC ACCCACGTAG ACGGTAACAA	1260
CCGCCACTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	1320
TCAGTATCCG GAAATATTG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	1380
GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG	1440
CGCCAGCATG GACAAATTCC GTCAGGCAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	1500
TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	1560
GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	1620
ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC	1680
TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA	1740
ACGCACATTT TCCC GTT CAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC	1800
GTCGCTCAGA TTGCGCGGCT GATGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC	1860
CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG	1920
CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG	1980
GATCACCAACA ATATTCA TAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC	2040
AAAATAGGGC AGTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG	2100
GTTCGTCATC ATCTTCTCC ATCTGGCGA CCTGATCGGT T	2141

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 403 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser			
1	5	10	15
Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln			
20	25	30	

- 54 -

Asn	Ala	Gly	Leu	Gly	Gly	Asn	Ser	Ala	Leu	Gly	Leu	Gly	Gly	Asn	
35						40						45			
Gln	Asn	Asp	Thr	Val	Asn	Gln	Leu	Ala	Gly	Leu	Leu	Thr	Gly	Met	Met
50						55						60			
Met	Met	Met	Ser	Met	Met	Gly	Gly	Gly	Gly	Leu	Met	Gly	Gly	Leu	
65						70					75			80	
Gly	Gly	Gly	Leu	Gly	Asn	Gly	Leu	Gly	Gly	Ser	Gly	Gly	Leu	Gly	Glu
85						90					95				
Gly	Leu	Ser	Asn	Ala	Leu	Asn	Asp	Met	Leu	Gly	Gly	Ser	Leu	Asn	Thr
100								105					110		
Leu	Gly	Ser	Lys	Gly	Gly	Asn	Asn	Thr	Thr	Ser	Thr	Thr	Asn	Ser	Pro
115								120					125		
Leu	Asp	Gln	Ala	Leu	Gly	Ile	Asn	Ser	Thr	Ser	Gln	Asn	Asp	Asp	Ser
130						135					140				
Thr	Ser	Gly	Thr	Asp	Ser	Ser	Thr	Ser	Asp	Ser	Ser	Asp	Pro	Met	Gln
145						150					155			160	
Leu	Leu	Lys	Met	Phe	Ser	Glu	Ile	Met	Gln	Ser	Leu	Phe	Gly	Asp	Gly
165						170					175				
Gln	Asp	Gly	Thr	Gln	Gly	Ser	Ser	Ser	Gly	Gly	Lys	Gln	Pro	Thr	Glu
180								185					190		
Gly	Glu	Gln	Asn	Ala	Tyr	Lys	Lys	Gly	Val	Thr	Asp	Ala	Leu	Ser	Gly
195						200					205				
Leu	Met	Gly	Asn	Gly	Leu	Ser	Gln	Leu	Leu	Gly	Asn	Gly	Gly	Leu	Gly
210						215					220				
Gly	Gly	Gln	Gly	Gly	Asn	Ala	Gly	Thr	Gly	Leu	Asp	Gly	Ser	Ser	Leu
225						230					235			240	
Gly	Gly	Lys	Gly	Leu	Gln	Asn	Leu	Ser	Gly	Pro	Val	Asp	Tyr	Gln	Gln
245								250					255		
Leu	Gly	Asn	Ala	Val	Gly	Thr	Ile	Gly	Met	Lys	Ala	Gly	Ile	Gln	
260							265					270			
Ala	Leu	Asn	Asp	Ile	Gly	Thr	His	Arg	His	Ser	Ser	Thr	Arg	Ser	Phe
275							280					285			
Val	Asn	Lys	Gly	Asp	Arg	Ala	Met	Ala	Lys	Glu	Ile	Gly	Gln	Phe	Met
290							295					300			
Asp	Gln	Tyr	Pro	Glu	Val	Phe	Gly	Lys	Pro	Gln	Tyr	Gln	Lys	Gly	Pro
305						310					315			320	
Gly	Gln	Glu	Val	Lys	Thr	Asp	Asp	Lys	Ser	Trp	Ala	Lys	Ala	Leu	Ser
325							330					335			
Lys	Pro	Asp	Asp	Asp	Gly	Met	Thr	Pro	Ala	Ser	Met	Glu	Gln	Phe	Asn
340						345					350				
Lys	Ala	Lys	Gly	Met	Ile	Lys	Arg	Pro	Met	Ala	Gly	Asp	Thr	Gly	Asn
355						360					365				

- 55 -

Gly Asn Leu Gln Ala Arg Gly Ala Gly Ser Ser Leu Gly Ile Asp
 370 375 380
 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
 385 390 395 400
 Gly Ala Ala

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1288 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCAAA	60
GAGGAATAACG TTATGAGTCT GAATAACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTCT	120
ATCGGCCGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTGCCAGAA TGCTGGTTG	180
GGTGGCAATT CTGCACTGGG GCTGGCGGC GGTAATCAAATGATACCGT CAATCAGCTG	240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG	300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA	360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA	420
GGCGGCAACA ATACCACCTTC AACAAACAAAT TCCCCGCTGG ACCAGGCCTGG GGGTATTAAC	480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC	540
CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGG	600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC	660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG	720
CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC	780
GGTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG	840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT	900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGCGA TCGGGCGATG	960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTGAAAGCGGAA GCCGCAGTAC	1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAAGCAGTGAGC	1080
AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC	1140

- 56 -

ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC	1200
GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA	1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT	1288

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 341 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met			
1	5	10	15
Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser			
20	25	30	
Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met			
35	40	45	
Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala			
50	55	60	
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Ile Glu Asp Val			
65	70	75	80
Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe			
85	90	95	
Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met			
100	105	110	
Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu			
115	120	125	
Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met			
130	135	140	
Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro			
145	150	155	160
Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe			
165	170	175	
Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile			
180	185	190	
Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly			
195	200	205	

- 57 -

Thr	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Ser	Ser	Phe	Ser	Asn	Asn	Ser	Ser
210						215						220			
Val	Met	Gly	Asp	Pro	Leu	Ile	Asp	Ala	Asn	Thr	Gly	Pro	Gly	Asp	Ser
225						230				235				240	
Gly	Asn	Thr	Arg	Gly	Glu	Ala	Gly	Gln	Leu	Ile	Gly	Glu	Leu	Ile	Asp
245						250					255				
Arg	Gly	Leu	Gln	Ser	Val	Leu	Ala	Gly	Gly	Gly	Leu	Gly	Thr	Pro	Val
260						265						270			
Asn	Thr	Pro	Gln	Thr	Gly	Thr	Ser	Ala	Asn	Gly	Gly	Gln	Ser	Ala	Gln
275						280						285			
Asp	Leu	Asp	Gln	Leu	Leu	Gly	Gly	Leu	Leu	Leu	Lys	Gly	Leu	Glu	Ala
290						295					300				
Thr	Leu	Lys	Asp	Ala	Gly	Gln	Thr	Gly	Thr	Asp	Val	Gln	Ser	Ser	Ala
305						310				315				320	
Ala	Gln	Ile	Ala	Thr	Leu	Leu	Val	Ser	Thr	Leu	Leu	Gln	Gly	Thr	Arg
									325		330			335	
Asn	Gln	Ala	Ala	Ala											
									340						

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1026 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAACCC	CGGCAATGGC	CCTTGTCTTG	60
GTACGTCTTG	AAGCCGAGAC	GAAGTGGCAGT	ACGTCGAGCA	AGGCGCTTCA	GGAAAGTTGTC	120
GTGAAGCTGG	CCGAGGAACT	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
AAACTGTTGG	CCAAGTGCAT	GGCCGCAGAT	GGCAAGGCCG	GCGGCCGTAT	TGAGGATGTC	240
ATCGCTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAACCTCGG	CGCGTCTGCG	300
GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
AAGTCGATGC	TCGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCAC	ACAGTTTCCC	480
AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAACCTCCT	TGATGGCGAC	540

- 58 -

GAAACGGCTG CGTTCCGTTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG	600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTCC	660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC	720
GGCAATAACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA	780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCAGACAC CGGTACGTG	840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG	900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT	960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA	1020
GCCTGAA	1026

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 344 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln			
1	5	10	15
Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser			
20	25	30	
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile			
35	40	45	
Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly			
50	55	60	
Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala			
65	70	75	80
Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser			
85	90	95	
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met			
100	105	110	
Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala			
115	120	125	
Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val			
130	135	140	

- 59 -

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
 145 150 155 160
 Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly
 165 170 175
 Gly Ala Gly Ala Gly Gly Ala Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
 Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
 195 200 205
 Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
 210 215 220
 Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
 225 230 235 240
 Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
 245 250 255
 Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln
 260 265 270
 Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
 275 280 285
 Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
 290 295 300
 Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
 305 310 315 320
 Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
 325 330 335
 Gln Ser Thr Ser Thr Gln Pro Met
 340

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1035 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCGG GTCTGCAGAA CCTGAACCTC	60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC	120
GAGAAGGACA TCCTAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC	180

- 60 -

GGCAACACCG	GTAACACCCG	CAACGCGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAAG	CGCTGATGCA	GCTGCTGGAA	360
GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCAGCGG	CAATGACAAG	420
GGCAACGGCG	TGGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCAGGCCAGGG	CGGCCTGGCC	480
GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	GCAGGCCAGCGG	TGCTGGCGCC	540
GGCGCGCGG	GTGGCGGTGT	CGGCAGGTGCT	GGTGGCGCGG	ATGGCGGCTC	CGGTGCGGGT	600
GGCCGAGGCG	GTGCGAACCG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGGCGAAC	660
GGCCCGCAGA	ACGCAGGCGA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
CAGGGCGGCC	TCACCGGGGT	GCTGAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
ATGATGCAGC	AAGGCGGCCT	CGGCGCGGC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	840
GGCAACGCCT	CGCCGGCTTC	CGGCGCGAAC	CCGGCGCGA	ACCAGCCCGG	TTCGGCGGAT	900
GATCAATCGT	CCGGCCAGAA	CAATCTGCAA	TCCCAGATCA	TGGATGTGGT	GAAGGAGGTC	960
GTCCAGATCC	TGCAGCAGAT	GCTGGCGCG	CAGAACGGCG	GCAGCCAGCA	GTCCACCTCG	1020
ACGCAGCCGA	TGTAA					1035

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Thr	Leu	Ile	Glu	Leu	Met	Ile	Val	Val	Ala	Ile	Ile	Ala	Ile	Leu	Ala
1					5				10				15		
Ala	Ile	Ala	Leu	Pro	Ala	Tyr	Gln	Asp	Tyr						
				20				25							

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS:

- 61 -

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1 5 10 15

Leu Leu Ala Met
20

- 62 -

WHAT IS CLAIMED:

1. A method of insect control for plants comprising:
 - 5 applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to a plant or plant seed under conditions effective to control insects on the plant or plants grown from the plant seed.
- 10 2. A method according to claim 1, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*,
 - 15 *Xanthomonas*, *Phytophthora*, and mixtures thereof.
- 20 3. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.
- 25 4. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.
5. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.
- 30 6. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

- 63 -

7. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

5 8. A method according to claim 2, wherein the hypersensitive response elicitor polypeptide or protein corresponds to a *Phytophthora* species.

10 9. A method according to claim 1, wherein the plant is selected from the group consisting of dicots and monocots.

15 10. A method according to claim 9, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, 20 pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

25 11. A method according to claim 9, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

30 12. A method according to claim 1, wherein plants are treated during said applying which is carried out by spraying, injection, or leaf abrasion at a time proximate to when said applying takes place.

35 13. A method according to claim 1, wherein plant seeds are treated during said applying which is

carried out by spraying, injection, coating, dusting, or immersion.

14. A method according to claim 1, wherein the
5 hypersensitive response elicitor polypeptide or protein
is applied to plants or plant seeds as a composition
further comprising a carrier.

15. A method according to claim 14, wherein
10 the carrier is selected from the group consisting of
water, aqueous solutions, slurries, and powders.

16. A method according to claim 14, wherein
the composition contains greater than 0.5 nM of the
15 hypersensitive response elicitor polypeptide or protein.

17. A method according to claim 14, wherein
the composition further contains additives selected from
the group consisting of fertilizer, insecticide,
20 fungicide, nematacide, and mixtures thereof.

18. A method according to claim 1, wherein the
hypersensitive response elicitor polypeptide or protein
is in isolated form.
25

19. A method according to claim 1, wherein the
hypersensitive response elicitor polypeptide or protein
is applied as bacteria which do not cause disease and are
transformed with a gene encoding the hypersensitive
30 response elicitor polypeptide or protein.

20. A method according to claim 1, wherein the
hypersensitive response elicitor polypeptide or protein
is applied as bacteria which cause disease in some plant
35 species, but not in those subjected to said applying, and

- 65 -

contain a gene encoding the hypersensitive response elicitor polypeptide or protein.

21. A method according to claim 1, wherein
5 said applying causes infiltration of the polypeptide or protein into the plant.

22. A method according to claim 1, wherein
said applying is effective to prevent insects from
10 contacting plants to which the hypersensitive response elicitor is applied.

23. A method according to claim 22, wherein
plants are treated during said applying.
15

24. A method according to claim 22, wherein
plant seeds are treated during said applying, said method further comprising:

planting the seeds treated with the
20 hypersensitive response elicitor in natural or artificial soil and
propagating plants from the seeds planted in the soil.

25. A method according to claim 1, wherein
said applying is effective to cause insects to depart from plants to which the hypersensitive response elicitor is applied.
25

26. A method according to claim 25, wherein
plants are treated during said applying.
30

27. A method according to claim 25, wherein
plant seeds are treated during said applying, said method further comprising:
35

planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

5 propagating plants from the seeds planted in the soil.

28. A method according to claim 1, wherein said applying is effective to kill insects proximate plants to which the hypersensitive response elicitor is applied.

29. A method according to claim 28, wherein plants are treated during said applying.

15 30. A method according to claim 28, wherein plant seeds are treated during said applying, said method further comprising:

20 planting the seeds treated with the hypersensitive response elicitor in natural or artificial soil and

propagating plants from the seeds planted in the soil.

31. A method according to claim 1, wherein
25 said applying is effective to interfere with insect larval feeding on plants to which the hypersensitive response elicitor is applied.

32. A method of insect control for plants
30 comprising:

providing a transgenic plant or plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and

growing the transgenic plants or transgenic plants produced from the transgenic plant seeds under conditions effective to control insects.

5 33. A method according to claim 32, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a pathogen selected from the group consisting of *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Phytophthora*, and mixtures
10 thereof.

15 34. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia chrysanthemi*.

20 35. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Erwinia amylovora*.

25 36. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas syringae*.

30 37. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Pseudomonas solanacearum*.

35 38. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from *Xanthomonas campestris*.

- 68 -

39. A method according to claim 33, wherein the hypersensitive response elicitor polypeptide or protein corresponds to that derived from a *Phytophthora* species.

5

40. A method according to claim 32, wherein the plant is selected from the group consisting of dicots and monocots.

10 41. A method according to claim 40, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, 15 turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

20

42. A method according to claim 40, wherein the plant is selected from the group consisting of rose, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

25

43. A method according to claim 32, wherein a transgenic plant is provided.

30 44. A method according to claim 32, wherein a transgenic plant seed is provided.

45. A method according to claim 32, further comprising:

- 69 -

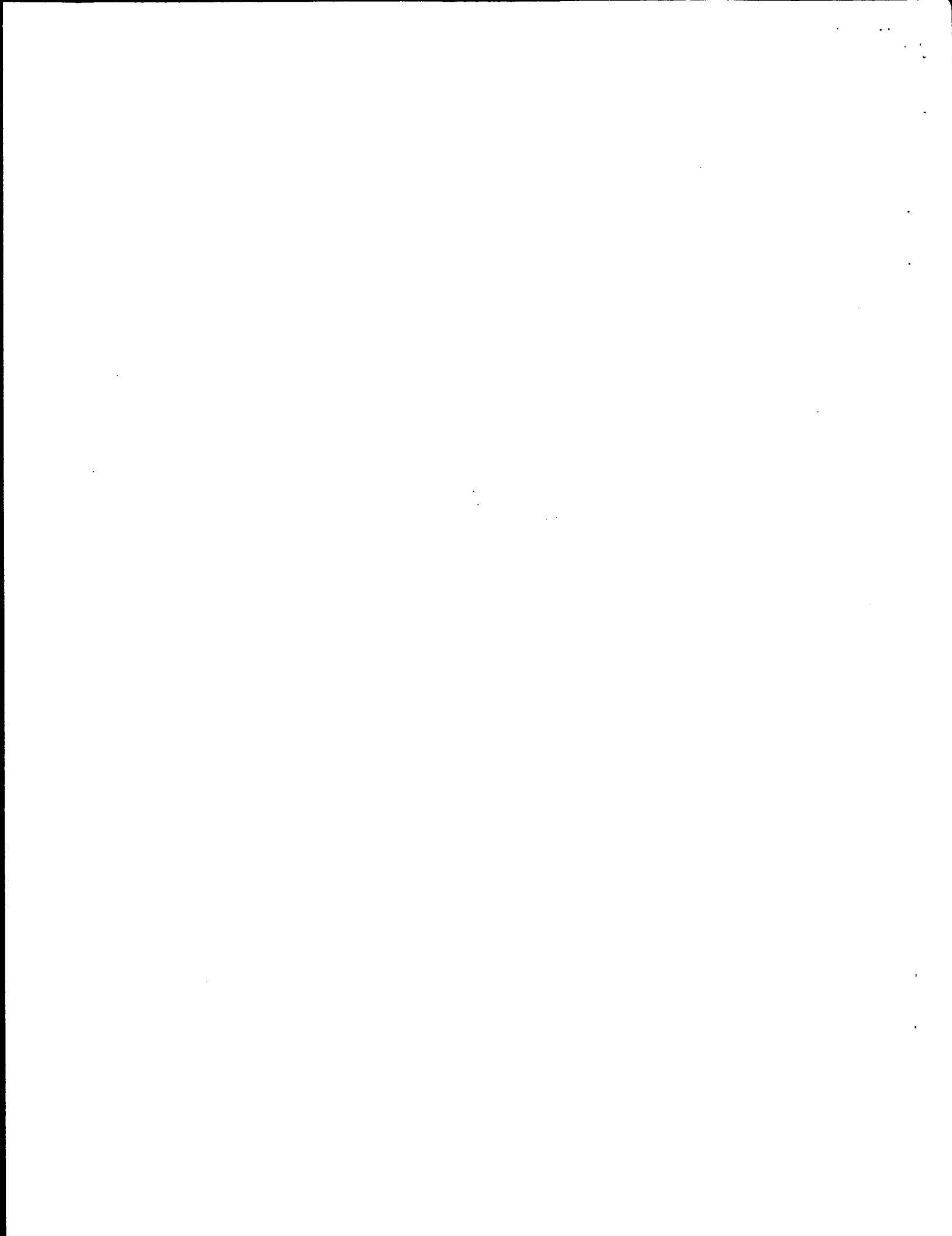
applying the hypersensitive response elicitor polypeptide or protein to the propagated plants to effect insect control.

5 46. A method according to claim 32, wherein said insect control prevents insects from contacting plants.

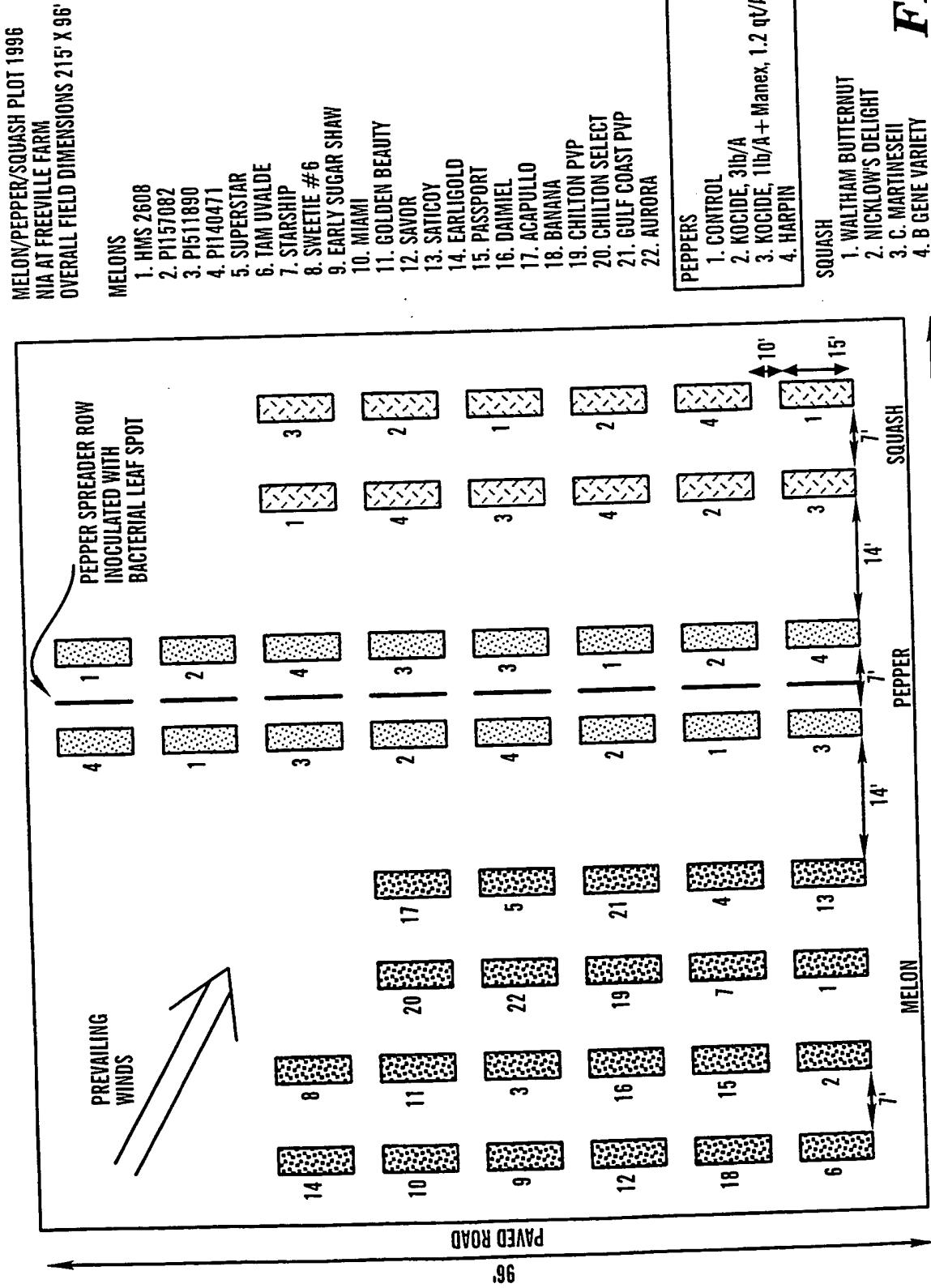
10 47. A method according to claim 32, wherein said insect control causes insects to depart from transgenic plants.

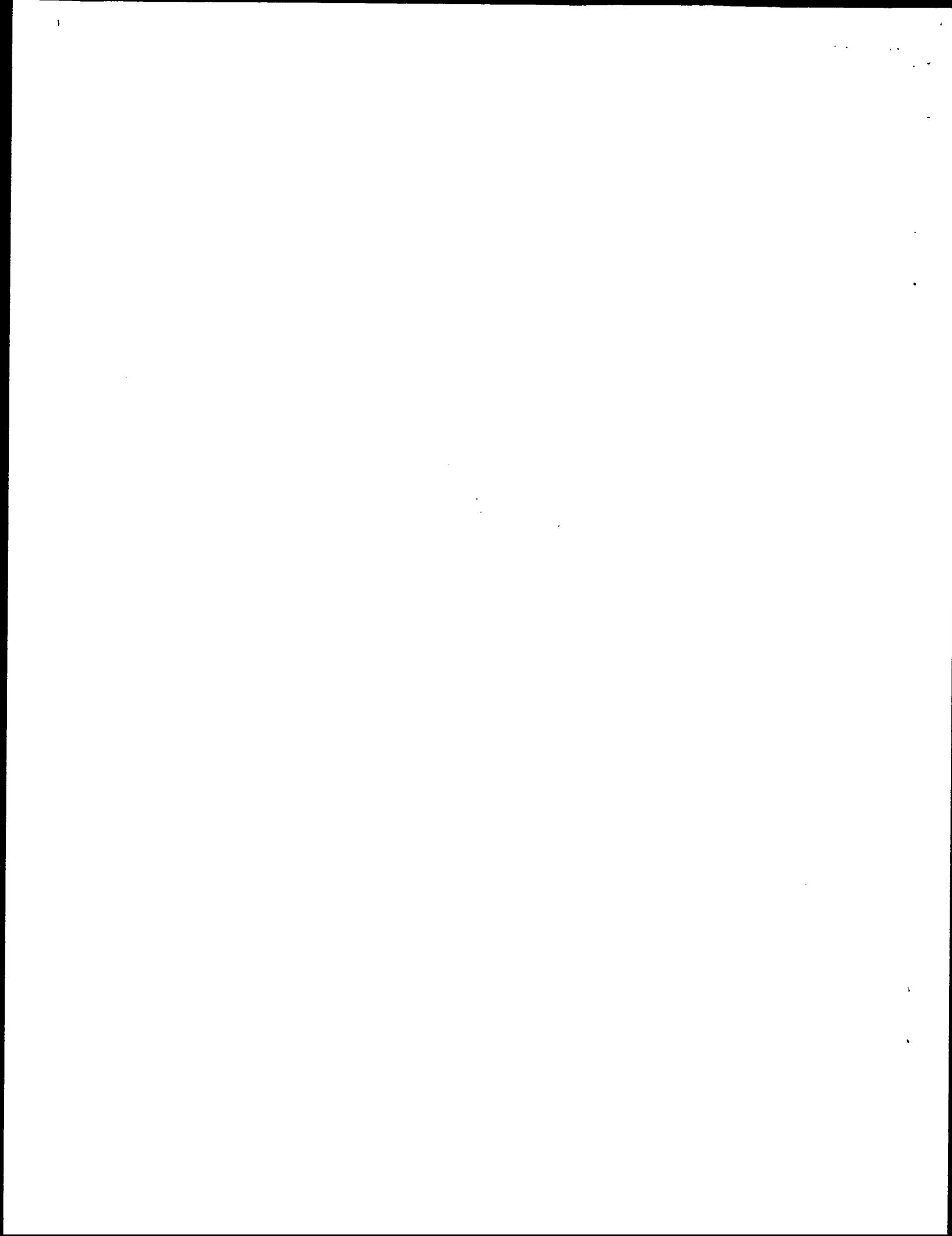
48. A method according to claim 32, wherein said insect control kills insects.

15 49. A method according to claim 32, wherein said insect control interferes with insect larval feeding on plants.

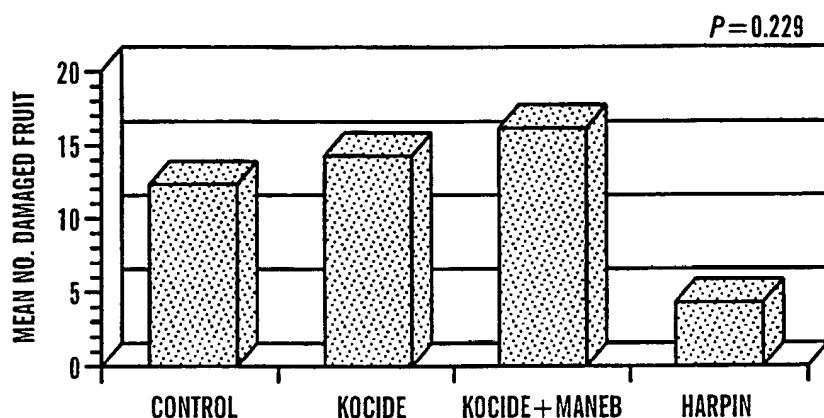
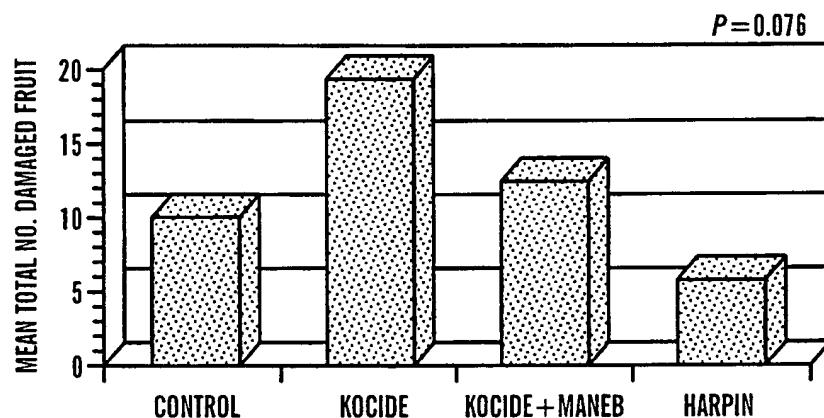
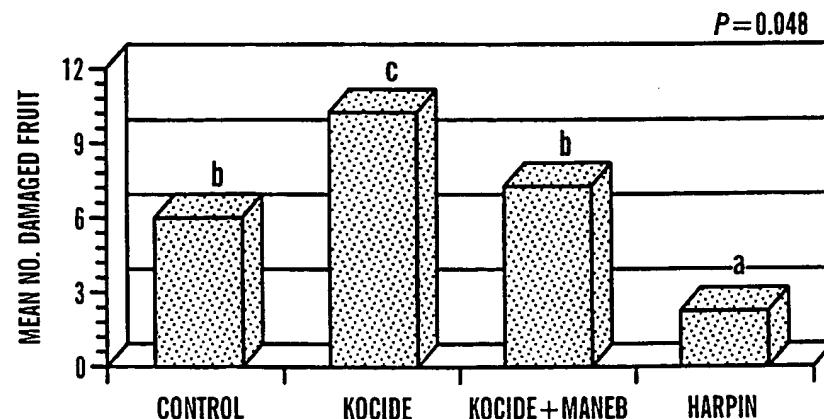


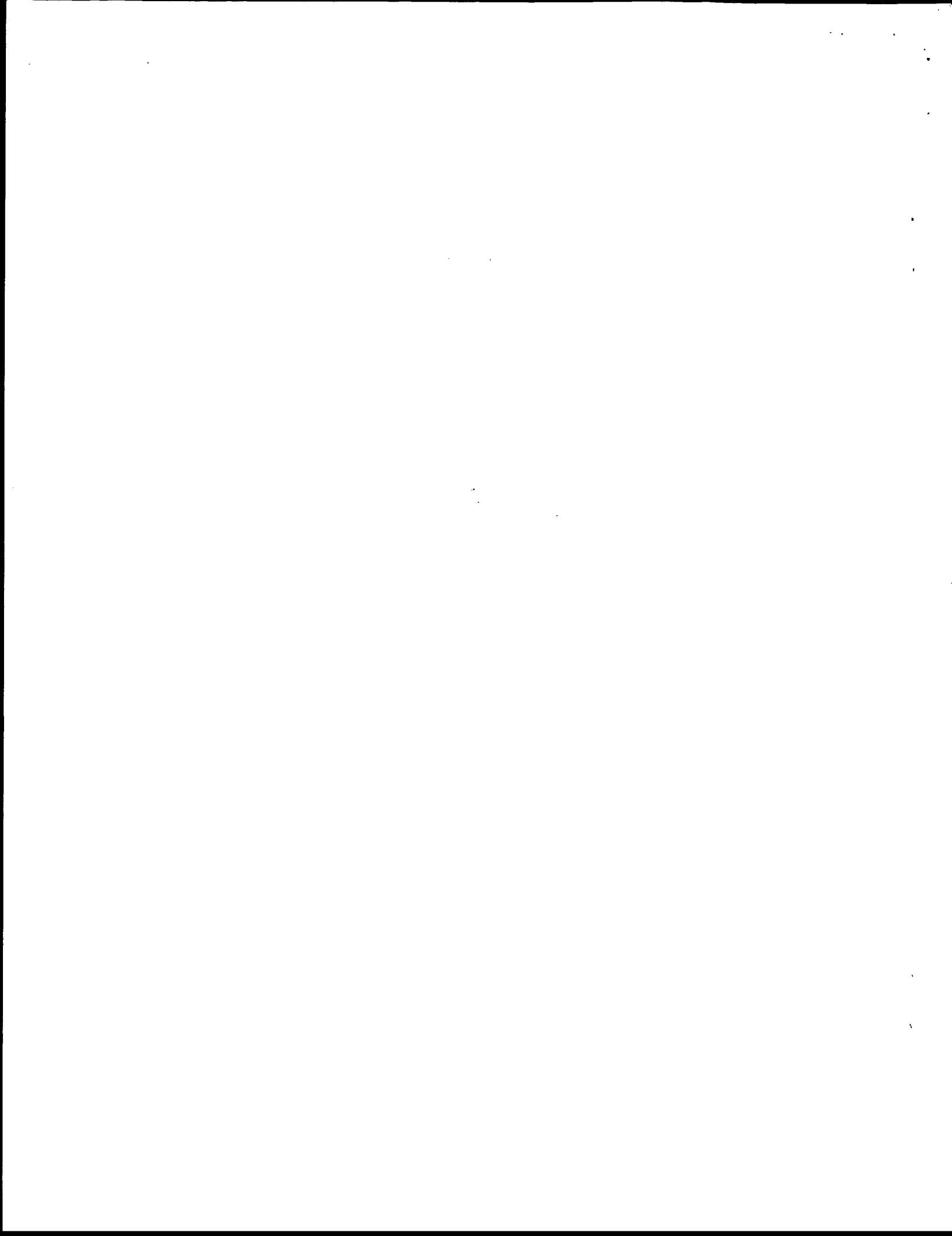
1/2

FIG. 1



2/2

**FIG. 2****FIG. 3****FIG. 4**
SUBSTITUTE SHEET (RULE 26)



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03604

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) A01G 1/00; A01H 1/00, 1/04, 5/10; C12N 5/04, 5/10
US CL :435/118; 800/200, 205, 250, 255

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/118; 800/200, 205, 250, 255

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	INBAR et al. Elicitors of plant defensive systems reduce insect densities and disease incidence. Journal of Chemical Ecology, January 1998, Vol. 24, No. 1, pages 135-149, see entire document.	1-49

Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- *A* document defining the general state of the art which is not considered to be of particular relevance
- *B* earlier document published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed
- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- *&* document member of the same patent family

Date of the actual completion of the international search

08 MAY 1998

Date of mailing of the international search report

23 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
IREM YUCEL
Telephone No. (703) 308-0916

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03604

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, DIALOG (AGRICOLA, CRIS, BIOSIS, SCISEARCH, MEDLINE), STN (CAPLUS)
Search Terms: HR, hypersensitive response, insect resist?, alkaloid?, phytoalexin?, salicylic acid, PR protein?, transgenic plant?, pathogen-induced resistance, inventor's names